REMARKS

With entry of the instant amendment, claims 20 –29 and 41 – 46 are pending. Claims 30 and 31 have been canceled. Original claims 20 - 24 and 26 - 29 have been amended, and claims 41 – 46 are new. New matter has not been introduced by the present amendment. Claims 1 – 19 and 32 – 40, directed to the non-elected invention, were previously canceled.

Claim Amendments.

The abbreviation KLG has been defined as 2-keto-L-gulonic acid and the phrase "as a carbon source" has been included with reference to KLG in amended claim 20. Minor clarifying corrections have been made to claims 21 and 23. The oxidative enzyme has been defined as having dehydrogenase activity, and the reducing enzyme has been defined as having reducatse activity. Moreover, the term "activity" has been omitted from claims 22 and 24 with reference to each enzymes type.

Claims 26, 27 28 29 now recite the corresponding yeast in italics.

New claims 41 – 43 are directed to recombinant *Candida blankii* or *Cryptococcus dimennae*, which are capable of using KLG as a carbon source to produce ASA or an ASA stereoisomer said yeast comprising either one or both of a heterologous nucleic acid encoding a glucose dehydrogenase and a heterologous nucleic acid encoding a 2,5 -diketo-L-gluconic acid (2,5-DKG) reductase wherein the yeast is capable of converting glucose to KLG and then utilizing the KLG as an intermediate to produce ASA or an ASA stereoisomer. Support is found at page 3, lines 9 and 20 – 28 of the disclosure.

New claims 44 – 46 are directed to recombinant *Candida blankii* or *Cryptococcus dimennae* yeast capable of using KLG as a carbon source to produce ASA or an ASA stereoisomer, said yeast comprising at least one heterologous nucleic acid encoding a L-sorbose dehydrogenase, a D-sorbitol dehydrogenase, a L-sorbosone dehydrogenase or a galactose dehydrogenase, wherein the yeast is capable of converting sorbitol to KLG and then utilizing the KLG to produce ASA or an ASA stereoisomer. Support is found at page 10, lines 3 – 9 of the disclosure.

Amendment to the Specification.

Applicant has requested that the specification be amended to reflect that the present application is a divisional application, and not a CIP, of parent application 09/205,874, now

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USP 6,358,715. Upon review of the file wrapper for this application, it was discovered that the Application Transmittal was confusing. Page 1 of the transmittal indicated Applicant was filing a divisional application. However, at page 2, point 13, Applicant requested amendment of the specification and inadvertently checked the continuation-in-part box as opposed to the divisional box. Applicant has herein requested that the first line of the specification be amended to correctly read that the application is a divisional application of parent application 09/205,874.

Claim Objections.

Claims 20, 24 and 30 have been objected to for the use of abbreviations without any explanation when they are recited for the first time and claims 27 - 31 have been objected to for reciting biological names that have not been recited in italics. Applicant has corrected these informalities in the amended claims. Specifically claims 20, 24 and 30 have been amended to recite the compounds and enzymes corresponding to the abbreviations used in the claims, and claims 27 - 31 have been amended to recite biological names in italics. Claim 30 has been canceled.

Rejections under 35 U.S.C. §112, second paragraph.

Claims 21 and 23 have been rejected for reciting the phrase "enzyme is a dehydrogenase activity" and "enzyme is a reductase activity"; claim 22 has been rejected for reciting the phrase "wherein said dehydrogenase activity includes"; claim 24 has been rejected for reciting the phrase "wherein said reductase activity includes"; claim 27 has been rejected for the phrase "wherein said yeast include Candida and Cryptococcus"; and claims 30 and 31 have been rejected due to lack of antecedent basis for the phrase "and said carbon source comprises".

Claims 21, 22, 23, 24, and 27 have been amended to more particularly point out the claimed subject matter. Applicant submits the amendment to said claims should obviate the rejections presented under section 112, second paragraph and that the claims are clear and definite. Claims 30 and 31 have been canceled.

Rejections under 35 U.S.C. §103(a).

Claims 20 - 30 have been rejected as unpatenable over Murakawa et al. (Agric. Biol. Chem. Vol 41(9):1799 - 1800); Hardy t al. (USP 4,945,052) and Anderson et al., (USP

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5,032,514). The Examiner states,

> "Murakawa et al. teach the production of ASA from non-recombinant yeast using a wide variety of sugars including glucose. However the yields appear to be low. Thus it appears that it was known in the art that 2,3-DKG occurs among yeast and that they are capable of producing ASA. Anderson et al., teach a metabolic pathway for engineering an increased production of ascorbic acid intermediates by using recombinant technology by transfer of genes responsible for the bioconversion of a six carbon sugar such as glucose to 2-KLG which is next oxidized to ascorbic acid using the very same enzymes taught in the instant application. ..... However the reference does not teach the utilization of yeast for the fermentative method or the bioconversion method. The reference does teach that the recombinant techniques can be used using any appropriate host cells..... Hardy et al teach the production if vitamin C precursors, 2,5-DKG, in genetically modified microorganisms including several bacteria, fungi and yeast... by transforming yeast host cells using a vector expressing the enzyme required for converting 2,5DKG to 2-KLG."

For a rejection under 35 U.S.C. §103(a) to be proper, the Examiner must show a) that each element of a claim is disclosed or suggested in the prior art, b) that the prior art provides the motivation to combine and modify the disclosures of the cited references to obtain the claimed invention and c) that the skilled artisan would have a reasonable expectation of success in obtaining the invention. Applicant asserts that these criteria have not been met because there is no disclosure or suggestion found in the cited references either alone or in combination of a recombinant yeast capable of using KLG as a sole carbon source for the production of ASA or an ASA stereoisomer wherein the yeast comprises a heterologous polynucleotide as claimed by Applicant. While arguably parts of Applicant's invention may be found in the cited references, the invention as a whole is not made obvious by the combination of references. Moreover, there is no motivation provided to modify the cited references to obtain the claimed invention.

Murakawa et al. suggest a hypothetical pathway for the biosynthesis of D-erythroascorbic acid (D-EasA) in the yeast *Candida utilis* starting from D-arabinose. Andersen et al. describe the overall process of converting a common metabolite such as glucose to 2-KLG in a single recombinant bacterial host cell. More specifically a polynucleotide encoding a reductase is introduced into the host microorganism. Hardy et al. concern the conversion of a carbon source such as glucose to 2-KLG in a single fermentation step. More specifically, Erwina is the host for a polynucleotide encoding a 2,5-DKG reductase. These cited references do not suggest a recombinant yeast which is capable of growing on KLG as a sole carbon source to produce ASA or an ASA stereoisomer wherein the

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yeast comprises a h terologous nucleic acid encoding an oxidative enzyme associated with the production of ASA or an ASA stereoisomer and/or a heterologous nucleic acid encoding a reducing enzyme associated with the production of ascorbic acid or an ascorbic acid stereoisomer in said yeast.

Additionally the cited references, taken alone or in any combination, do not render new independent claim 41 unpatentable. Claim 41, and those claims dependent thereon, are directed to recombinant *Candida blankii* or *Cryptococcus dimennae* that are capable of growing on 2-KLG as a carbon source to produce ASA or an ASA stereoisomer but which have been grown in media including glucose and further include a heterologous nucleic acid encoding a glucose dehydrogenase and/or a heterologous nucleic acid encoding a 2,5 -diketo-L-gluconic acid (2,5-DKG) reductase wherein the *Candida blankii* or *Cryptococcus dimennae* converts the glucose to KLG and then utilizes the KLG to produce ASA or an ASA stereoisomer.

The Examiner has also rejected claim 31 as unpatentable over the combined references of Murakawa et al., Hardy et al., Anderson et al. as applied to claims 20 – 29 above and further in view of Saito et al. While claim 31 has been canceled, Applicant asserts for the record that that original claim 31 was not rendered obvious by the combination of references. Applicant additionally asserts new claim 44 is patentable over the cited references. The Murakawa et al., Hardy et al. and Anderson et al. references are discussed above. While Saito et al. may disclose the cloning of genes coding for L-sorbose dehydrogenase, the reference alone nor in combination suggests or teaches a yeast, particularly a *Candida blankii* or *Cryptococcus dimennae* that is capable of growing on KLG to produce ASA or an ASA stereoisomer, which is further engineered to include a polynucleotide encoding a L-sorbose dehydrogenase.

Applicant believes the instant claims are in condition for allowance and an early allowance for claims 20 – 29 and 41 – 46 is kindly requested.

Respectfully submitted,

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